CORECEPTOR TROPISM ASSAYS (Updated January 10, 2011)

Panel's Recommendations:

- ***Coreceptor tropism assay should be performed whenever the use of a CCR5 inhibitor is being considered (AI).***
- ***Coreceptor tropism testing might also be considered for patients who exhibit virologic failure on a CCR5 inhibitor (CIII).***

Rating of Recommendations: A = Strong; B = Moderate; C = Optional
Rating of Evidence: I = data from randomized controlled trials; II = data from well-designed nonrandomized trials or observational cohort studies with long-term clinical outcomes; III = expert opinion

HIV enters cells by a complex process that involves sequential attachment to the CD4 receptor followed by binding to either the CCR5 or CXCR4 molecules and fusion of the viral and cellular membranes *[1]*. CCR5 inhibitors (i.e., maraviroc [MVC]), prevent HIV entry into target cells by binding to the CCR5 receptor *[2]*. Phenotypic and, to a lesser degree, genotypic assays have been developed that can determine the coreceptor tropism (i.e., CCR5, CXCR4, or both) of the patient's dominant virus population. One assay (*Trofile*, Monogram Biosciences, Inc., South San Francisco, CA) was used to screen patients who were participating in studies that formed the basis of approval for MVC, the only CCR5 inhibitor currently available. Other assays are under development and are currently used primarily for research purposes or in clinical situations in which the *Trofile* assay is not readily available.

Background

The vast majority of patients harbor a CCR5-utilizing virus (R5 virus) during acute/recent infection, which suggests that the R5 variant is preferentially transmitted compared with the CXCR4 (X4) variant. Viruses in many untreated patients eventually exhibit a shift in coreceptor tropism from CCR5 to either CXCR4 or both CCR5 and CXCR4 (i.e., dual- or mixed-tropic; D/M-tropic). This shift is temporally associated with a more rapid decline in CD4 T-cell counts *[3-4]*, although whether this shift is a cause or a consequence of progressive immunodeficiency remains undetermined *[1]*. Antiretroviral (ARV)-treated patients who have extensive drug resistance are more likely to harbor detectable X4- or D/M-tropic variants than untreated patients who have comparable CD4 T-cell counts *[5]*. The prevalence of X4- or D/M-tropic variants increases to more than 50% in treated patients who have CD4 counts <100 cells/mm^3 *[5-6]*.

Phenotypic Assays

There are now at least two high-throughput phenotypic assays that can quantify the coreceptor characteristics of plasma-derived virus. Both involve the generation of laboratory viruses that express patient-derived envelope proteins (i.e., gp120 and gp41). These pseudoviruses are either replication competent (*Phenoscript* assay, VIRalliance, Paris, France) or replication defective (*Trofile* assay, Monogram Biosciences, Inc.) *[7-8]*. These pseudoviruses then are used to infect target cell lines that express either CCR5 or CXCR4. In the *Trofile* assay, the coreceptor tropism of the patient-derived virus is confirmed by testing the susceptibility of the virus to specific CCR5 or CXCR4 inhibitors *in vitro*. The *Trofile* assay takes about 2 weeks to perform and requires a plasma HIV RNA level ≥1,000 copies/mL.

The performance characteristics of these assays have evolved. Most, if not all, patients enrolled in premarketing clinical trials of MVC and other CCR5 inhibitors were screened with an earlier, less sensitive version of the *Trofile* assay *[7]*. This earlier assay failed to routinely detect low levels of CXCR4-utilizing variants. As a consequence, some patients enrolled in these clinical trials harbored low, undetectable levels of CXCR4-utilizing viruses at baseline and exhibited rapid virologic failure after initiation of a CCR5 inhibitor *[9]*. This assay has since been revised and is now able to detect lower levels of CXCR4-utlizing viruses. *In vitro*, the assay can detect CXCR4-utilizing clones with 100% sensitivity when those clones make up 0.3% of the population *[10]*. Although this more sensitive assay has had limited use in prospective clinical trials, it is now the only one that is commercially available. For unclear reasons, a minority of samples cannot be successfully phenotyped with either generation of the *Trofile* assay. In patients with plasma HIV-1 RNA below the limit of detection, coreceptor usage can be determined from proviral DNA obtained from peripheral blood mononuclear cells; however, the clinical utility of this assay remains to be determined *[11]*.

Genotypic Assays

Genotypic determination of HIV-1 coreceptor usage is based on sequencing the V3-coding region of HIV-1 *env*, the principal determinant of coreceptor usage. A variety of algorithms and bioinformatics programs can be used to predict coreceptor usage from the V3 sequence. When compared to the phenotypic assay, genotypic methods show high specificity (~90%) but only modest sensitivity (~50%–70%) for the presence of a CXCR4-utilizing virus. Given these performance characteristics, these assays may not be sufficiently robust to completely rule out the presence of an X4 or D/M variant *[12]*.

Recent studies in which V3 genotyping was performed on samples from patients screening for clinical trials of MVC suggest that genotyping performed as well as phenotyping in predicting the response to MVC *[13-14]*. On the basis of these data, accessibility, and cost, European guidelines currently favor genotypic testing for determining coreceptor usage. An important caveat to these results is that the majority of patients who received MVC were first shown to have R5 virus by a phenotypic assay (*Trofile*). Consequently, the opportunity to assess treatment response to MVC in patients whose virus was considered R5 by genotype but D/M or X4 by phenotype was limited to a relatively small number of patients. It is also important to note that the genotyping approaches used in these studies are not routinely available from clinical laboratories in the United States at this time.

Given the uncertainty regarding the genotypic assays and fewer logistical barriers to obtaining a phenotype in the United States than elsewhere, the Panel recommends that a phenotype be used as the preferred coreceptor tropism screening test in the United States.

Use of Coreceptor Tropism Assays in Clinical Practice

Coreceptor tropism assays should be used whenever the use of a CCR5 inhibitor is being considered **(AI)**. Coreceptor tropism testing might also be considered for patients who exhibit virologic failure on MVC (or any CCR5 inhibitor) **(CIII)**.

Other potential clinical uses for the tropism assay are for prognostic purposes or for assessment of tropism prior to starting antiretroviral therapy (ART), in case a CCR5 inhibitor is required later (e.g., in a regimen change for toxicity). Currently, sufficient data do not exist to support these uses.

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